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Immunotherapy of rheumatic diseases – practice and prospects

Gabrielle Kingsley, Gabriel Panayi and Jerry Lanchbury

Present treatments for rheumatic diseases are both toxic to patients and largely ineffective in modifying the disease process. This report, based on a meeting recently held in London, investigates how far recent molecular and immunological advances can be converted into more effective, less toxic and, above all, more specific therapies.*

The greatest obstacle to developing specific effective therapy for rheumatoid arthritis (RA) has been lack of understanding of aetiology. Nevertheless, based on present knowledge, a pathogenetic model can be constructed (Fig. 1) in which rheumatoid antigen(s) is presented in the context of a limited range of major histocompatibility complex (MHC) class II structures to the disease-promoting $CD4^+$ T cells that become activated[1,2]. These, in turn, activate other cells including macrophages, B cells, other T cells and synoviocytes to release effector molecules such as cytokines, growth factors, antibodies and degradative enzymes. This leads to synovial inflammation and proliferation which cause joint destruction. It is likely that this immune activation will trigger a variety of immunoregulatory mechanisms but these are as yet ill-defined. Whether the critical MHC–antigenic peptide–T-cell receptor interactions occur in central lymphoid organs or whether this activation occurs primarily *in situ* after migration of T cells to the synovium is not known. In either case migration of T cells must play an important role in generating synovitis. Each component of this pathogenetic pyramid is a potential target for immunotherapeutic intervention.

Therapy directed against cytokines

There is a consensus that the monokines interleukin 1 (IL-1), IL-6, granulocyte–macrophage colony-stimulating factor (GM-CSF) and tumour necrosis factor α (TNF-α) are present within the joint in rheumatoid arthritis patients at high concentrations but there is considerable controversy about T-cell-derived cytokines such as IL-2, IL-4 and gamma-interferon (IFN-γ)[1,3]. The role of IL-7, IL-8, IL-9 and IL-10 is even more uncertain although, interestingly, IL-8 has recently[4] been shown to be elevated in RA synovial fluid compared with other inflammatory arthritides.

Several cytokine-based therapeutic approaches are being used experimentally but, as yet, none has been applied to humans. Strategies include anti-cytokine monoclonal antibodies, soluble cytokine receptor proteins and specific cytokine inhibitors, notably the naturally-occurring IL-1 receptor antagonist (IL-1ra)[5]. One potential practical problem in the therapeutic use of these small molecular weight proteins is the inordinately high doses required; for example, in endotoxic shock in rabbits a total of 100 mg kg^{-1} IL-1ra was required to achieve a good therapeutic effect[6]. However, the most important question regarding cytokine intervention in rheumatic disease lies not in its technical feasibility but in the likely effect of interfering with only one cytokine within what is undoubtedly a very complex network. It seems highly improbable that a single cytokine holds the key to RA synovitis.

What of other targets for therapy? Macrophages are possible targets[3] but have not yet been investigated. Autoantibodies, notably rheumatoid factor, were at one time popular

*The First International Symposium on the Immunotherapy of the Rheumatic Diseases was held in London, UK on 20–22 February 1991 and was organized by Gabrielle Kingsley, Gabriel Panayi and Jerry Lanchbury.

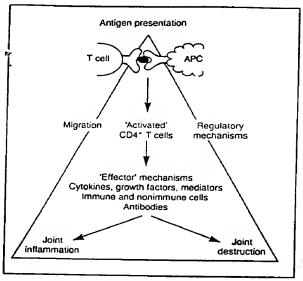

Fig. 1. *A model for the pathogenesis of rheumatoid arthritis.*

targets for therapy but the lack of effect of plasmapheresis on RA synovitis provides fairly conclusive evidence against them. T-cell therapy is currently favoured.

Therapy directed towards T cells

There is overwhelming evidence that supports a central position for the T cell in rheumatoid synovitis: immunohistological studies of RA synovium demonstrate perivascular aggregates of activated $CD4^+$ T cells closely applied to antigen-presenting cells[7]; T cells specific for the inducing agent from animals with experimental arthritides, such as adjuvant arthritis and streptococcal cell wall arthritis, can transfer disease; in human inflammatory arthritides of known cause (reactive arthritis) T cells specific to the inciting antigen are found in the joint. The critical role of T cells in RA is corroborated by the striking association of particular HLA class II alleles with the disease, given that these molecules restrict the presentation of antigenic peptides to $CD4^+$ T cells[8]. Most convincingly, early attempts at T-cell-directed immunotherapy in humans, such as thoracic duct drainage, total lymphoid irradiation and lymphocytapheresis, while neither practical nor safe as routine therapy, resulted in disease remission. The recent beneficial use of cyclosporin A, which, primarily, interferes with T-cell function, in RA further supports their importance.

Several studies of monoclonal antibodies directed against T-cell targets have been undertaken in RA patients. A monoclonal antibody (RFT2) against the T-cell activation antigen CD7 (Ref. 9) (shown to be effective in transplantation) had little clinical benefit in RA, although $CD7^+$ T cells disappeared both from blood and synovium. A better clinical result was obtained using Campath-6, a monoclonal antibody against the IL-2 receptor: two out of three patients improved for at least three months[10]. However, the most exciting results in RA come from the, by now extensive, studies using anti-CD4 antibody. In Europe, about 100 patients have been treated with murine and, more recently, chimeric human–mouse monoclonal anti-CD4 antibodies in open studies[11,12]. Approximately 60% of the patients show a significant clinical improvement, similar to that seen with methotrexate or cyclosporin A, and lasting on average from two to three months. No serious toxicity has been observed and only low titre anti-mouse antibodies develop; thus retreatment is possible. Double-blind placebo-controlled multicentre studies are now underway.

Disappointingly, the mode of action of anti-CD4 antibodies remains unclear. An anti-idiotypic response does not appear to develop and there is no long-term immunosuppression: in most patients, only a transient fall in $CD4^+$ cells is seen. Is this evidence for reprogramming of the immune system as proposed by Waldmann[13]?

Another agent used in treatment is anti-CD5 antibody coupled to ricin (although it is not established that ricin is necessary for its action). Promising results have been achieved, especially in early disease, but controlled studies are awaited[14]. The process of T-cell migration should also be considered as a target. No studies in RA have yet been conducted but anti-lymphocyte function-associated molecule 1 (LFA-1) antibody has been used in human bone marrow transplantation.

Although the anti-CD4 antibody looks a promising treatment, it is a nonspecific mode of immunointervention directed against all CD4 cells. Of greater interest is the possibility of therapy directed against the apex of the pyramid (Fig. 1), that is the specific T cells, antigenic peptides and MHC molecules involved in RA.

T-cell vaccination

In T-cell vaccination, first developed in animal models by Irun Cohen[15], subpathogenic doses of disease-promoting T cells are injected into the skin, inducing regulatory T cells that downregulate activity. For example, a T-cell clone, A2b, derived from an animal with adjuvant arthritis, could transfer disease to naive animals. This A2b clone could protect against arthritis and suppress established disease if it was both activated and chemically fixed or irradiated. Vaccinated animals develop a cell-mediated immune response against the A2b T-cell clone. Interestingly, in some animal models the anti-vaccine response appears to amplify a pre-existing immunoregulatory T-cell response.

Since the eliciting antigen in most rheumatic diseases is unknown, the production of specific T-cell lines or clones to be used as vaccines is clearly impossible. However, in adjuvant arthritis it is possible to expand the regulatory T cells by antigen-nonspecific stimulation of T cells from central lymphoid organs[15]. Based on this work, activated T cells have been expanded from the joints of patients with RA and have been used as vaccines. These trials are underway in Leiden, Mainz and London. The initial concern of all investigators has been to establish the safety of the procedure. Approximately 15 patients with RA have been treated to date without any significant side effects but it is too early to consider clinical effectiveness.

The T-cell repertoire and immunization with T-cell receptor peptides

By its very nature, the technique of T-cell vaccination requires a preparation unique to each patient. If the relevant antigens on the T cell could be identified then a standard vaccine could be developed. One leading candidate for the stimulating moiety is the T-cell receptor (TCR). For example, in experimental allergic encephalomyelitis (EAE), disease-inducing T cells express a limited repertoire of TCRs, predominantly $V_\alpha 2V_\beta 8$; in the Lewis rat. A 21

amino acid peptide, including the complementarity-determining region (CDR) 2 of $V_\beta 8$, was effective in protecting against and treating rats with EAE[16]. Vandenbark and colleagues suggest that these effects are due to the augmentation of a pre-existing immunoregulatory network. Confirmation of these exciting findings is eagerly awaited.

The major problem in the application of this technique to the treatment of RA is that it requires that the disease is caused by T cells with a restricted TCR usage. Conflicting evidence exists for oligoclonal αβ TCR use in RA. This may reflect the diversity of material studied, which includes uncultured and *in vitro* IL-2 expanded synovial fluid and membrane T cells, examined mainly by Southern blotting and C_β probe hybridization (M. Steinmetz and Y. Uematsu, unpublished). Recently anchor and inverse polymerase chain reaction (PCR), coupled with sequencing and V and J region oligonucleotide screening of αβ TCR libraries, have been applied to joint and peripheral blood T cells. This technique provides a sensitive and unselective estimate of the T-cell repertoire and has been exploited[17] to analyse peripheral blood, synovial fluid and synovial membrane T cells in HLA-DR4$^+$ RA patients. Initial analysis shows a skewing towards $V_\beta 2.1$ use in synovial T cells. In one patient the $V_\beta 2.1$ segment was preferentially associated with $J_\beta 2.3$, which may indicate clonal expansion. It is not clear whether such extreme and site-directed distortion of repertoire relates to chronic ongoing joint disease or to short-term infection, since it is known that $V_\beta 2.1$ populations are expanded by bacterial superantigens.

MHC-binding peptides

Genetic and epidemiological studies suggest that the HLA component of susceptibility to RA is a relatively conserved pentapeptide sequence that locates to the putative α helical region bordering the peptide-binding groove of RA-associated HLA-DR alleles[8]. Recent studies have reinforced the view that this epitope is preferentially associated with chronic RA (particularly disease involving extra-articular manifestations) and is not equally prevalent in all populations. Modelling the contact sites in susceptibility versus nonsusceptibility alleles over this helical region suggests that variability of peptide contact sites may be critical in the disease process.

The binding of specific peptide by MHC molecules represents an attractive target for therapeutic disruption by peptide blockade providing that soluble MHC blockers can be exchanged for prebound peptide *in vivo* without inducing novel immunogenicity. These criteria have been satisfied in model experimental systems (reviewed in Ref. 18) but, in animal models of autoimmune disease, MHC-blocking peptides have been successfully used only for prophylaxis and not for treatment.

In experimental models, controversy exists about whether selective blocking peptides should be sequence modifications of the original disease-inducing peptide, or high-affinity peptides that are unrelated in sequence. The *in vivo* effects of the former are difficult to interpret since they may cause deviation rather than blockage of the immune response. In terms of a therapy for RA, it is likely that screening of randomly-derived peptides for binding to suitable HLA-DR molecules represents a reasonable first step since convincing candidate peptides for sequence modification have yet to be described.

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